

The amendment of Claims 21 and 22 changes the description of analysis methods from "consists" to "comprises." Support for these changes can be found, for example, on page 20 of the specification. Here, methods of analysis are described in terms that contemplate the methods delineated in the claims, as well as other methods. Specifically, the specification reads, "As regards the analysis of restriction fragments, it *may* consist in counting said fragments and/or in determining their length." (Specification page 20, lines 4-6, emphasis added.) Furthermore, the next paragraph begins with the statement, "*Another way of analyzing* the restriction fragments resulting from the enzymatic digestion of the genome of the mycobacterium as described above consists in bringing said fragments into contact with at least one appropriate probe, covering *for example* the duplicated region, under hybridization conditions so as to then identify the number and size of the fragments which have hybridized." (Specification page 20, lines 19-21, emphasis added.) These statements demonstrate that analysis of the fragments can include various techniques and methods.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

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Dated: November 20, 2001

By: 

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**Appendix to Amendment of November 20, 2001**

Please amend the claims as follows:

1. (Amended) A [Nucleotide] nucleotide or polynucleotide [sequences] sequence deleted from the genome of *M. bovis* BCG/*M. bovis* and present in the genome of *M. tuberculosis* or [conversely chosen from] a nucleotide or polynucleotide sequence of the following ORFs and genes: Rv2346c, Rv2347c, Rv2348c, *plcC*, *plcB*, *plcA*, Rv2352c, Rv2353c, Rv3425, Rv3426, Rv3427c, Rv3428c, Rv1964, Rv1965, *mce3*, Rv1967, Rv1968, Rv1969, *lprM*, Rv1971, Rv1972, Rv1973, Rv1974, Rv1975, Rv1976c, Rv1977, *ephA*, Rv3618, Rv3619c, Rv3620c, Rv3621c, Rv3622c, *lpqG*, *cobL*, Rv2073c, Rv2074, Rv2075, *echA*, Rv0223c, RvD1-ORF1, RvD1-ORF2, Rv2024c, *plcD*, RvD2-ORF1, RvD2-ORF2, RvD2-ORF3, or Rv1758.

2. (Amended) The nucleotide or polynucleotide sequences as claimed in claim 1 grouped together in nucleotide regions RD5 to RD10 and RvD1 and RvD2 according to the following distribution:

[-] (A) RD5: Rv2346c, Rv2347c, Rv2348c, *plcC*, *plcB*, *plcA*, Rv2352c, Rv2353c[.];

[-] (B) RD6: Rv3425, Rv3426, Rv3427c, Rv3428c[.];

[-] (C) RD7: Rv1964, Rv1965, *mce3*, Rv1967, Rv1968, Rv1969, *lprM*, Rv1971, Rv1972, Rv1973, Rv1974, Rv1975, Rv1976c, Rv1977[.];

[-] (D) RD8: *ephA*, Rv3618, Rv3619c, Rv3620c, Rv3621c, Rv3622c, *lpqG*[.];

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- [-] (E) RD9: *cobL*, Rv2073c, Rv2074, Rv2075c[.];
- [-] (F) RD10: *echA1*, Rv0223c[.];
- [-] (G) RvD1: RvD1-ORF1, RvD1-ORF2, Rv2024c; and
- [-] (H) RvD2: *plcD*, RvD2-ORF1, RvD2-ORF2, RvD2-ORF3, Rv1758.

3. (Amended) A method for the discriminatory detection and identification of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample, comprising [the following steps]:

[a)](A) [isolation of] isolating the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample[.];

[b)](B) [detection of] detecting the DNA sequences of the mycobacterium present in said biological sample[.]; and

[c)](C) [analysis of] analyzing said sequences with the nucleotide and polynucleotide sequences as claimed in claim 1 [or 2].

4. (Amended) The method as claimed in claim 3, [in which] wherein the detection of the mycobacterial DNA sequences is carried out using nucleotide sequences complementary to said DNA sequences.

5. (Amended) The method as claimed in claim 3, [or 4, in which] wherein the detection of the mycobacterial DNA sequences is carried out by [amplification of these] amplifying the sequences using primers.

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6. (Amended) The method as claimed in claim 5, [in which] wherein the primers have a nucleotide sequence chosen from the group comprising SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, SEQ ID No. 16, SEQ ID No. 17, and SEQ ID No. 18 [with] wherein:

(A) the pair SEQ ID No. 1/SEQ ID No. 2 is specific for RD4;

[-] (B) the pair SEQ ID No. 3/SEQ ID No. 4 is specific for RD5[,];

[-] (C) the pair SEQ ID No. 5/SEQ ID No. 6 is specific for RD6[,];

[-] (D) the pair SEQ ID No. 7/SEQ ID No. 8 is specific for RD7[,];

[-] (E) the pair SEQ ID No. 9/SEQ ID No. 10 is specific for RD8[,];

[-] (F) the pair SEQ ID No. 11/SEQ ID No. 12 is specific for RD9[,];

[-] (G) the pair SEQ ID No. 13/SEQ ID No. 14 is specific for RD10[,];

[-] (H) the pair SEQ ID No. 15/SEQ ID No. 16 is specific for RvD1[,]; and

[-] (I) the pair SEQ ID No. 17/SEQ ID No. 18 is specific for RvD2[,];

8. (Amended) A method for the discriminatory detection and identification of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample, comprising [the following steps]:

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[a)](A) bringing the biological sample to be analyzed into contact with at least one pair of primers as defined in claim 6 [or 7], the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand[.];

[b)](B) [amplification of] amplifying the DNA of the mycobacterium[.]; and

[c)](C) [visualization of] visualizing the amplification of the DNA fragments.

9. (Amended) A kit for the discriminatory detection and identification of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample comprising [the following elements]:

[a)](A) at least one pair of primers as defined in claim 6 [or 7.];

[b)](B) [the] reagents necessary to carry out a DNA amplification reaction[.]; and

[c)](C) optionally, the necessary components, which make it possible to verify or compare the sequence [and/or], the size of the amplified fragment, or both the sequence and the size of the amplified fragment.

10. (Amended) [The use of at least one pair of primers as defined in claim 6 or 7 for the amplification of] A method of amplifying a DNA sequence from *M. bovis* BCG/*M. bovis* or *M. tuberculosis* comprising hybridizing at least one of the pair of primers of claim 6 to the DNA sequence.

11. (Amended) A product of expression of all or part of [the] a nucleotide or polynucleotide [sequences] sequence deleted from the genome of *M. bovis* BCG/*M.*

*bovis* and present in *M. tuberculosis* or [conversely as defined in] a product of expression of all or a part of an ORF or gene of claim 1.

12. (Amended) A method for the discriminatory detection *in vitro* of antibodies directed against *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample, comprising [the following steps]:

[a)](A) bringing the biological sample into contact with at least one product as defined in claim 11, and

[b)](B) detecting the antigen-antibody complex formed.

13. (Amended) A method for the discriminatory detection of a vaccination with *M. bovis* BCG or an infection by *M. tuberculosis* in a mammal, comprising [the following steps]:

[a)](A) [preparation of] preparing a biological sample containing cells[, more particularly cells of the immune system of said mammal and more particularly still T cells],

[b)](B) [incubation of] incubating the biological sample [of step a)] with at least one product as defined in claim 11, and

[c)](C) [detection of] detecting a cellular reaction indicating prior sensitization of the mammal to said product, [in particular] wherein the cellular reaction is cell proliferation [and/or], synthesis of proteins [such as gamma-interferon], or both cell proliferation and synthesis of proteins such as gamma interferon.

14. (Amended) A kit for the *in vitro* diagnosis of an *M. tuberculosis* infection in a mammal optionally vaccinated beforehand with *M. bovis* BCG comprising:

[a)](A) a product as defined in claim 11[.];

[b)](B) where appropriate, [the] reagents for the constitution of the medium suitable for the immunological reaction[.];

[c)](C) [the] reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction[.];

[d)](D) where appropriate, a reference biological sample (negative control) free of antibodies recognized by said product[.]; and

[e)](E) where appropriate, a reference biological sample (positive control) containing a predetermined quantity of antibodies recognized by said product.

15. (Amended) A mono- or polyclonal antibody, or its chimeric fragments or antibodies, [characterized in that they are] wherein the antibodies or fragments are capable of specifically recognizing a product as defined in claim 11.

16. (Amended) A method for the discriminatory detection of the presence of an antigen of *M. bovis* BCG/ *M. bovis* or *M. tuberculosis* in a biological sample comprising [the following steps]:

[a)](A) bringing the biological sample into contact with an antibody as claimed in claim 15[.]; and

[b)](B) detecting the antigen-antibody complex formed.

17. (Amended) A kit for the discriminatory detection of the presence of an antigen of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample comprising [the following steps]:

[a)](A) an antibody as claimed in claim 15[.];

[b)](B) [the] reagents for constituting the medium suitable for the immunological reaction[.]; and

[c)](C) [the] reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

18. (Amended) An immunological composition, [characterized in that it comprises] comprising at least one product as defined in claim 11, and a pharmaceutically compatible vehicle.

19. (Amended) [A] The vaccine of claim 18, [characterized in that it comprises] further comprising [at least one product as defined in claim 11 in combination with a pharmaceutically compatible vehicle and, where appropriate,] one or more [appropriate] immunity adjuvants.

20. (Amended) A method for the discriminatory detection and identification of *M. bovis* BCG or *M. tuberculosis* in a biological sample comprising the following steps:

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[-] (A) [digestion] digesting with *HindIII*, of at least part of the genome of the mycobacterium present in a biological sample to be analyzed[,] ; and

[-] (B) [analysis of the] analyzing restriction fragments thus obtained.

21. (Amended) The method as claimed in claim 20, [in which] wherein the analysis of the restriction fragments [consists in] comprises counting said fragments [and/or in], determining [their] the length of said fragments, or both counting said fragments and determining the length of said fragments.

22. (Amended) The method [Method] of detection as claimed in [either of claims] claim 20, [and 21, in which] wherein the analysis of the restriction fragments [consists in] comprises bringing [them] the fragments into contact with at least one probe under stringent hybridization conditions and [in] identifying the [fragment parts or fragment] fragments hybridized.

23. (Amended) [A] The method as claimed in claim 22, wherein [characterized in that] the probe is obtained by amplification of the genomic DNA with primers chosen from the group SEQ ID No. 31, SEQ ID No. 32, SEQ ID No. 33, or SEQ ID No. 34 with the pair:

[-] (A) SEQ ID No. 31/SEQ ID No. 32 specific for DU1; or

[-] (B) SEQ ID No. 33/SEQ ID No. 34 specific for DU2.

25. (Amended) The method as claimed in claim 20, [characterized in that] wherein the analysis of the fragments obtained comprises [are amplified] amplification with

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primers and sequencing, wherein the primers are chosen from the group SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22, SEQ ID No. 23, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 35, SEQ ID No. 36, SEQ ID No. 37, and SEQ ID No. 38, wherein

(A) SEQ ID No. 19, SEQ ID No. 20/SEQ ID No. 21 are specific for JDU1;

(B) SEQ ID No. 22, SEQ ID No. 24/SEQ ID No. 23, SEQ ID No. 25 are specific for JDU2A;

(C) SEQ ID No. 26/SEQ ID No. 27, SEQ ID No. 28 are specific for JDU2B

(D) SEQ ID No. 36, SEQ ID NO. 37, SEQ ID No. 38 are specific for DU1.

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